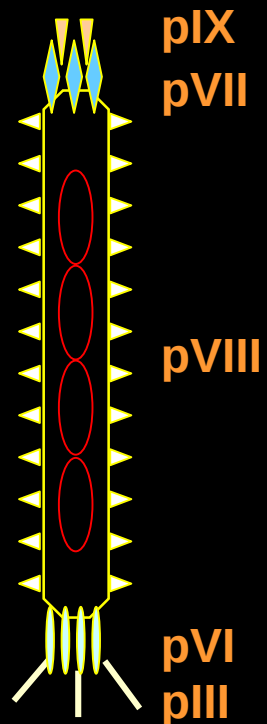
Phage Display

Introduction I

- Combinatorial display of peptides or proteins by fusion to phage coat proteins (George P. Smith, Science 228:1335, 1985).
- Powerful tool to identify partners of protein-protein interactions.
- One of the most established techniques to generate lead molecules in drug discovery.
- Based on the fact that most protein-protein interactions involve direct contact of very small numbers of amino acids.



M13

Phage Display

Introduction II

Types of Phage Display Libraries

- Non-lytic phage display derived from M13 bacterial filamentous phage

  pIII, pVIII, pVI

  Random peptides

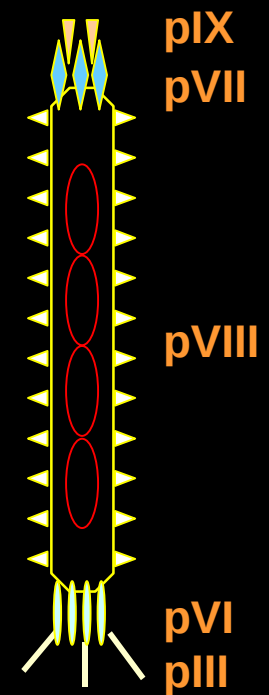
  Custom-designed peptides or proteins

  Antibodies (single chain Fv fusion to pIII) (Nature 348:552; Immunotech. 4:1).

- Lytic Phage display

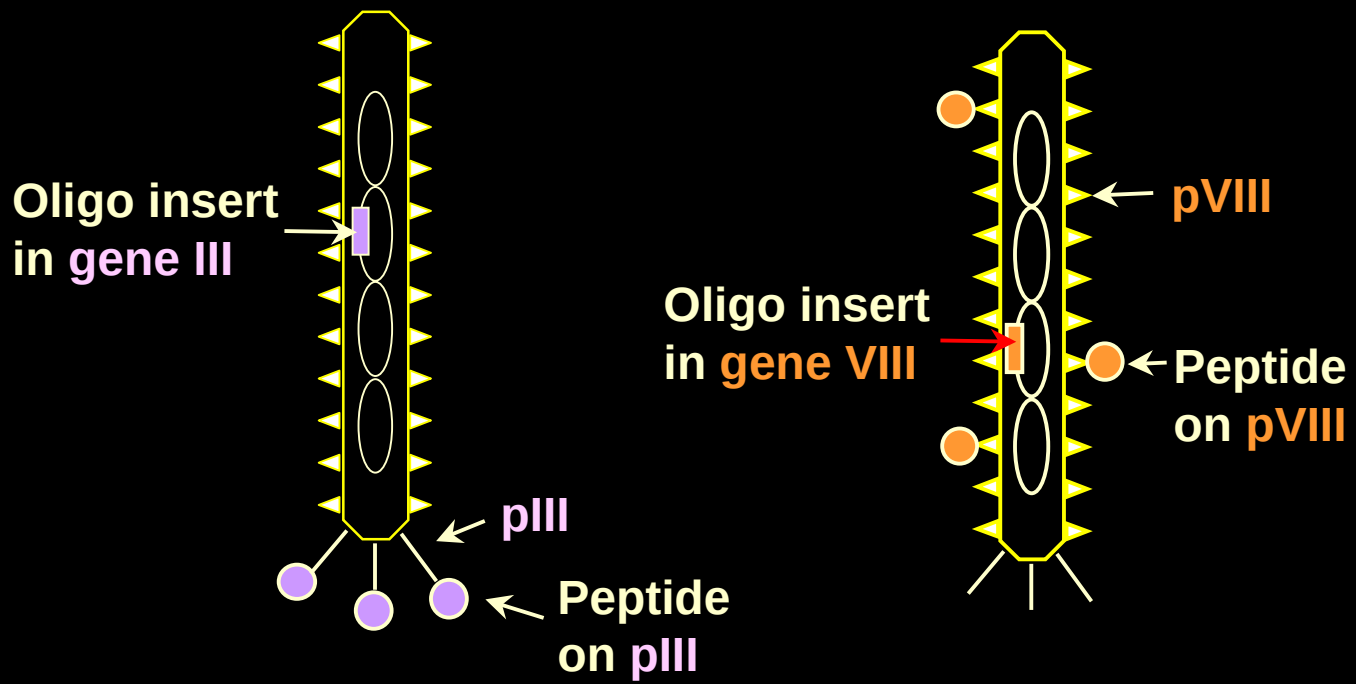
  λ-, T7-, T4-based

  Preferred for cDNA library display (J. Imm. Meth. 231:39; Nature Biotech. 19:1193).



M13

Peptide Display on M13 Filamentous Phage



Key Feature:

Direct linkage between a displayed peptide and its coding sequence. In traditional libraries, such as λ expression libraries, there is no such linkage.

The value of the linkage is that vast display libraries can be interrogated by a process of affinity selection, no filter screening, no microarray.

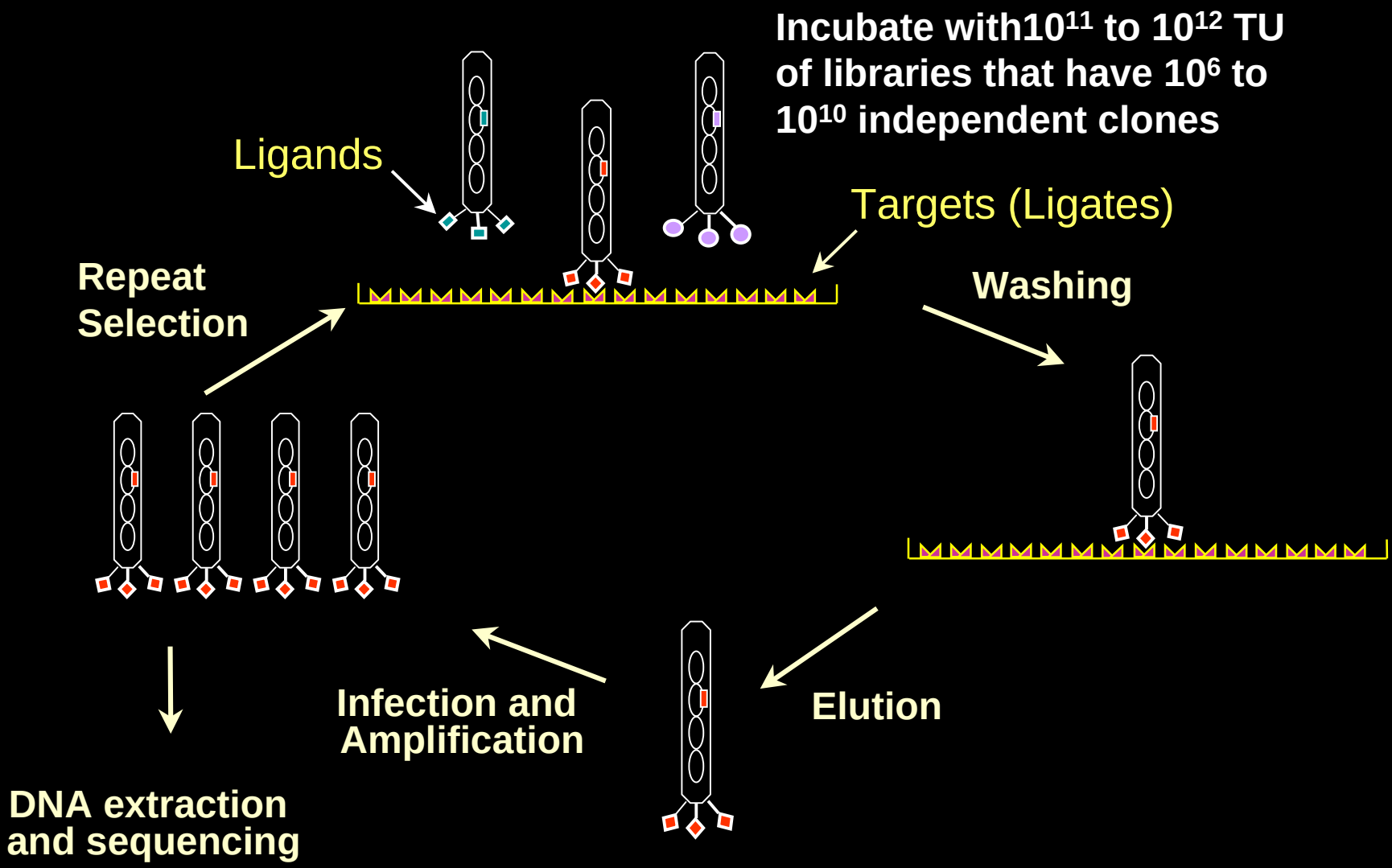
Other Features:

1) Vast number of random peptides can be displayed. Libraries containing 10^6 to 10^{10} independent clones can be readily constructed.
2) Out of frame mutants are replication defective.
3) Non-lytic, tetracycline-resistant, can be propagated like plasmid (quantitated in transducing units, TU, not PFU).

Targets of Phage Display

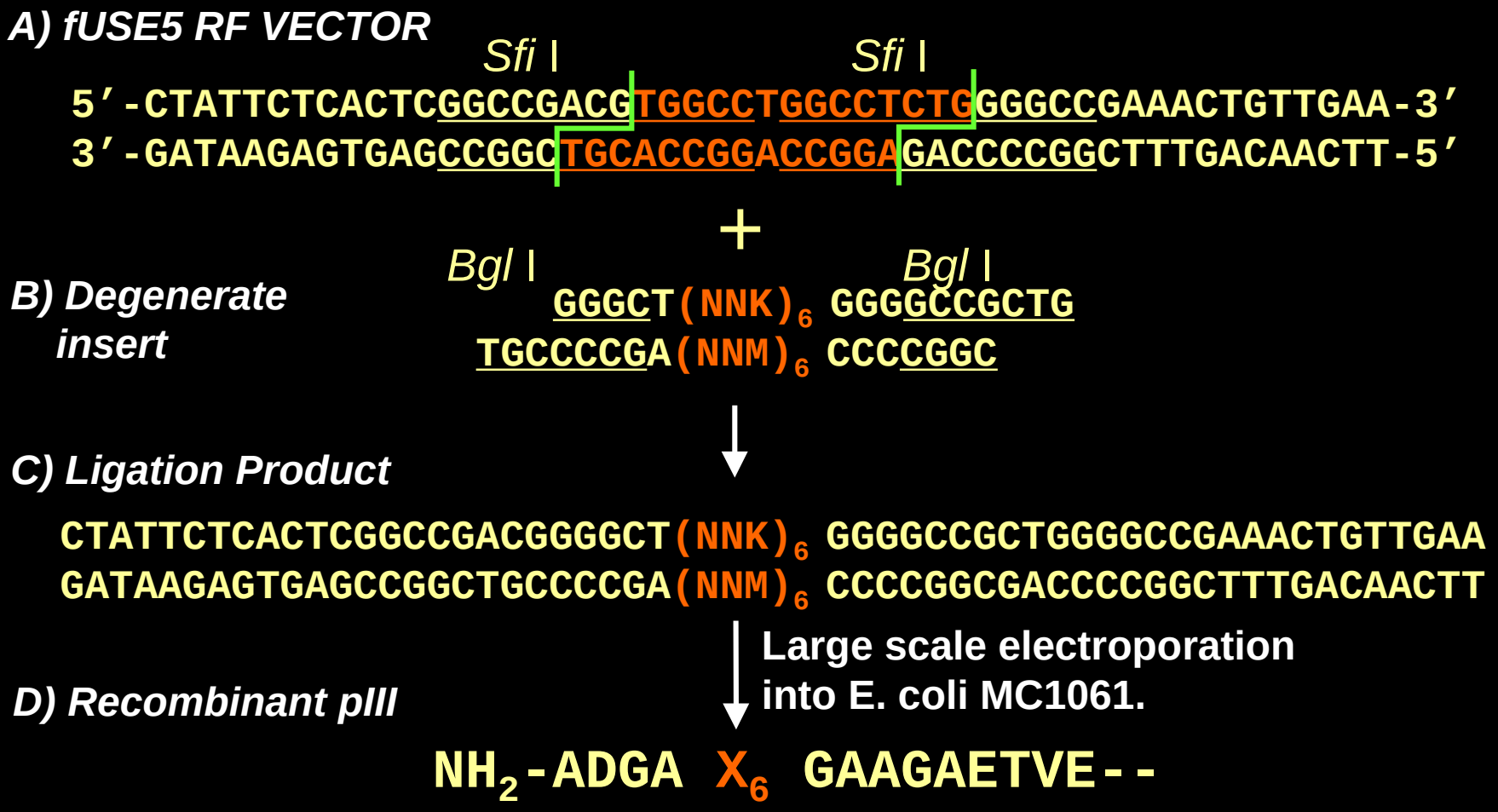
- Molecules immobilized on solid support.
- Cell surface proteins in vitro.
- Organ-specific endothelial cell markers in vivo (Pasqualini and Ruoslahti, Nature 380:364).

Biopanning: Affinity-Selection of Ligands From Phage Display Libraries



Key Determinant: Quality of Libraries

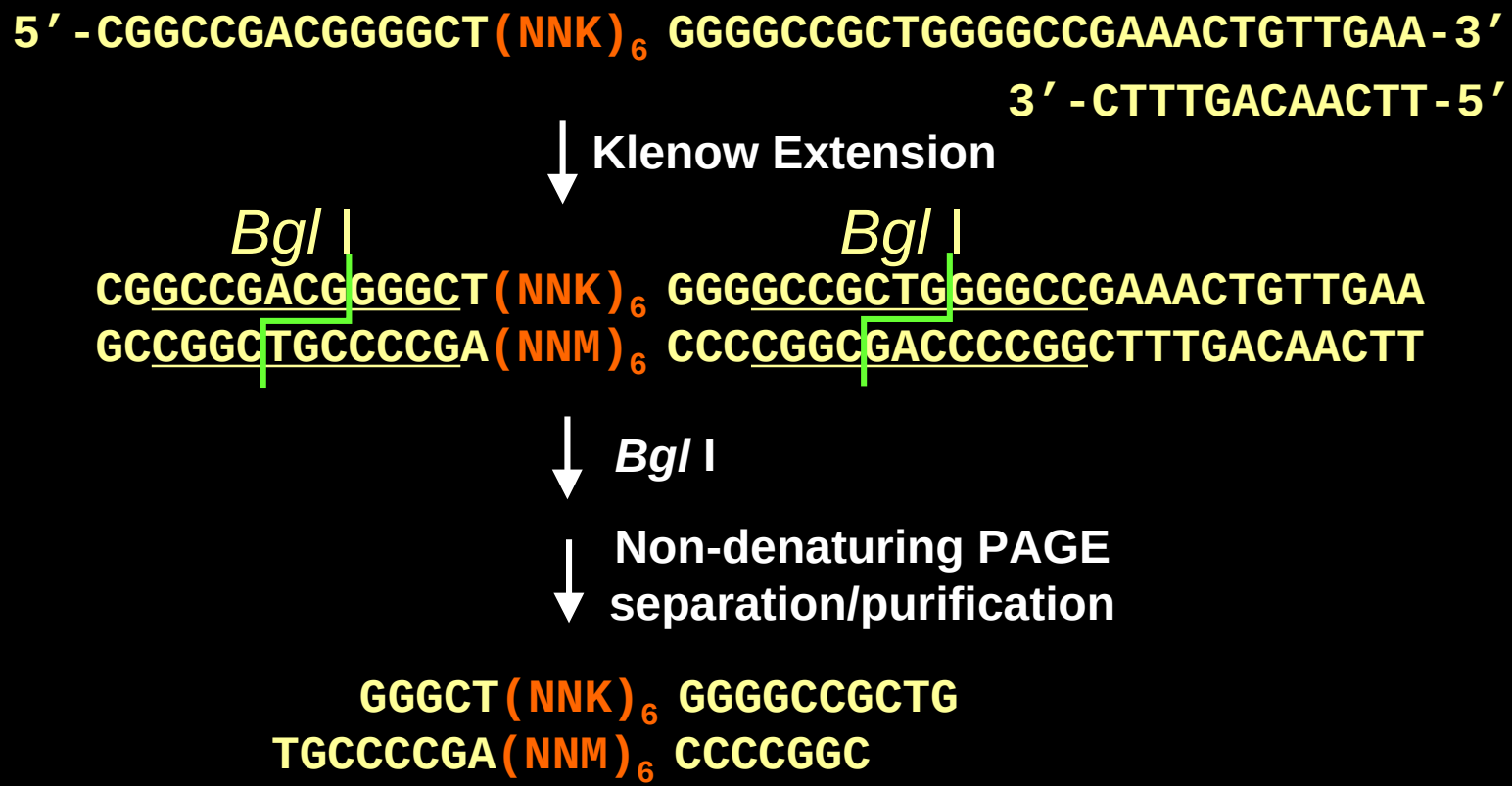
Construction of Random Peptide Phage Display Libraries



K=T,G; M=A,C; N=G,A,T,C. X=any amino acid.

Science 249:386

Insert Preparation for Construction of Random Peptide Phage Display Libraries



Method Enzym. 245:346

Types of Random Peptide Libraries Constructed

Oligo Inserts	Amino Acid
$(NNK)_{10}$	X_{10}
$*TGT(NNK)_n\ TGT$	CX_nC
$(NNK)_2TGT(NNK)_{14}TGT(NNK)_2$	$X_2CX_{14}CX_2$
$TGT(NNK)_9$	CX_9
$(NNK)_2TGT(NNK)_{18}$	X_2CX_{18}

*n=3-7

Applications and Expected Outcomes

1) Biopanning on recombinant proteins.

   14-3-3ζ-binding peptide (R18), and crystallographic structure of R18-14-3-3ζ complex.

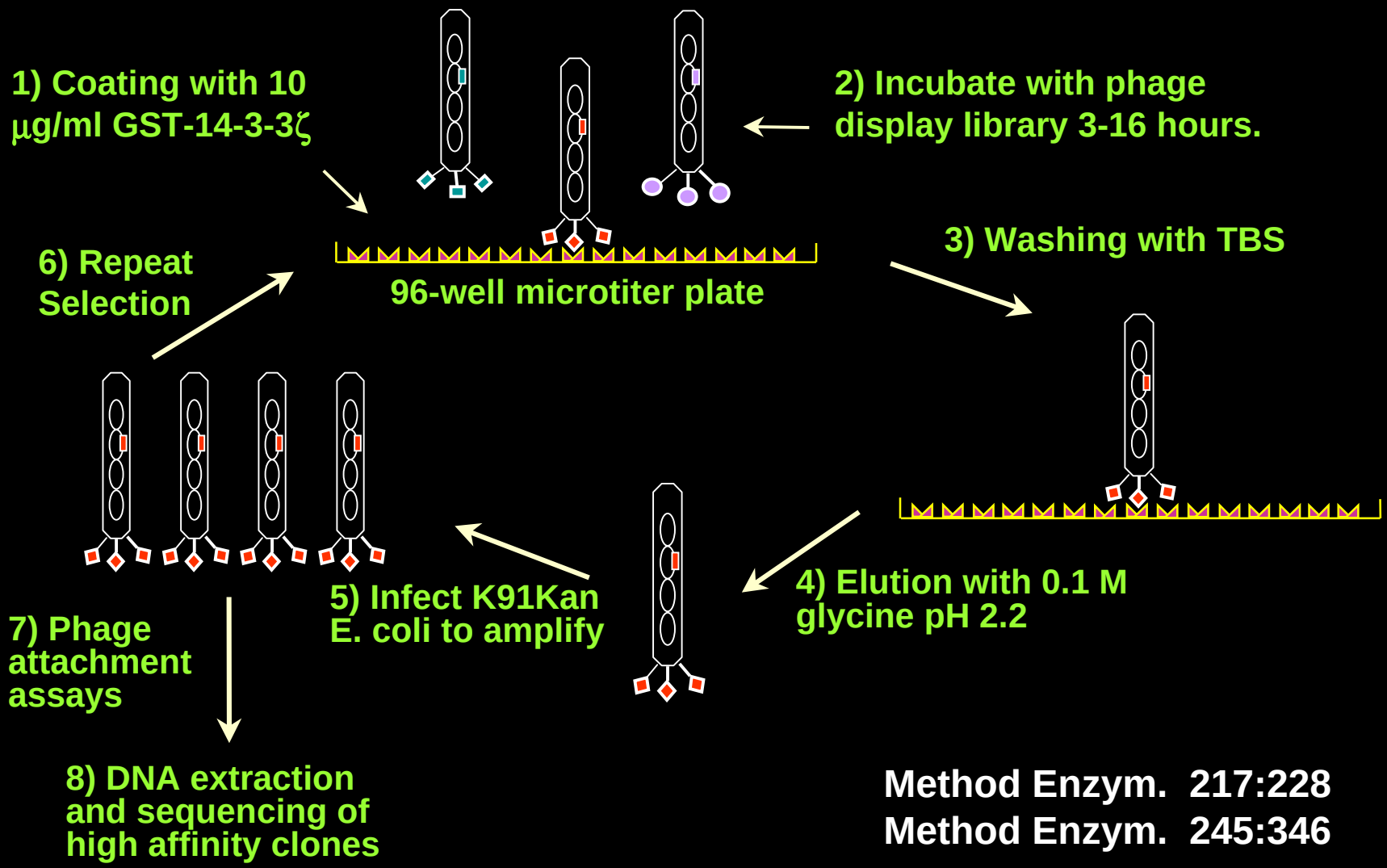
2) Biopanning on cells, a new strategy.

   Differentiated vs undifferentiated cells, or transfected vs untransfected cells.

3) Toward peptide-based therapy in vivo.

   Inhibition of tumor growth by an EphA2 kinase binding peptide fused to IgG.

Searching Peptide Ligands on Immobilized GST-14-3-3ζ



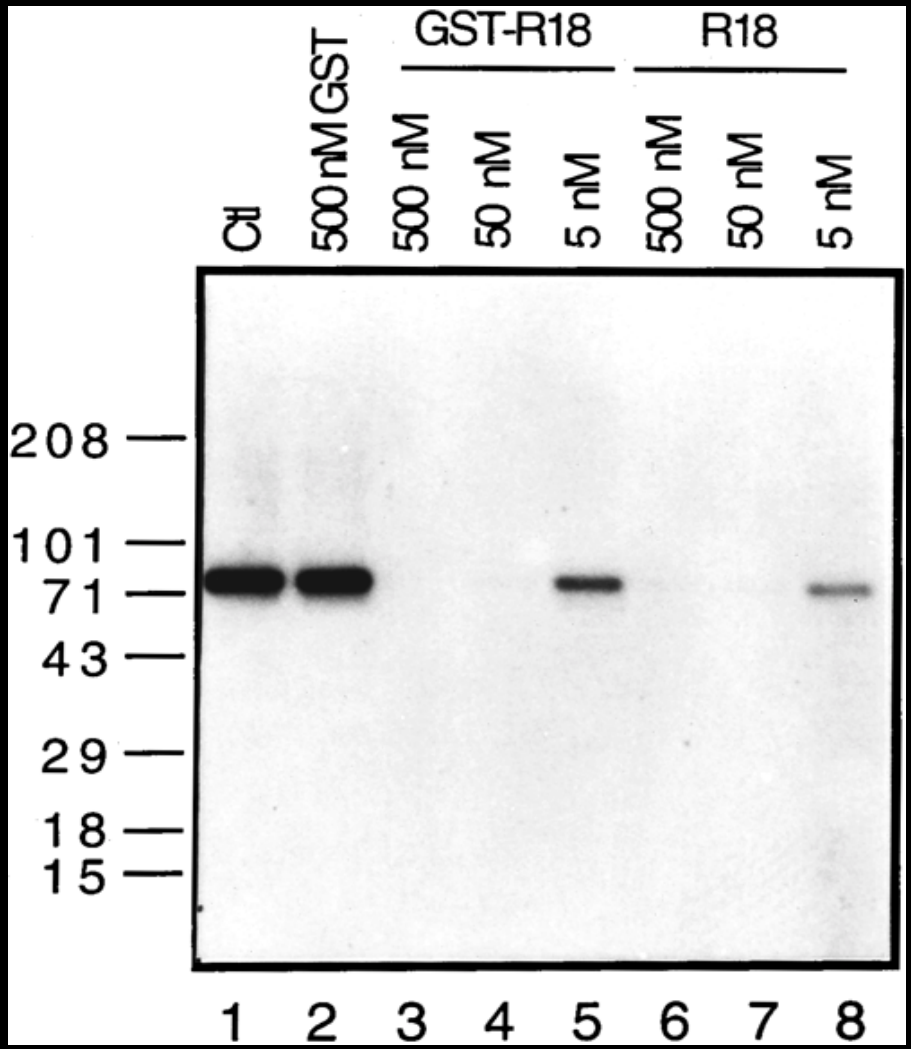
Method Enzym. 217:228
Method Enzym. 245:346

14-3-3ζ Ligands Displayed on pIII of M13 Phage after Four Rounds of Affinity-Selection

Clone	Sequence	Frequency	Library
R18	PHCVPRDLS**WLDL**EANMCLP	18	$X_2CX_{14}CX_2$
C43	VTCGSIAEYG**WLDL**AAACSS	1	$X_2CX_{14}CX_2$
C12	PRCMQTSY**WMDG**LQPESCKG	1	$X_2CX_{14}CX_2$
C48	RNCWGNIPLTSSSVERLCDAR	1	X_2CX_{18}
C03	RVCAAPESRLFRGMPLGCDD	1	$X_2CX_{14}CX_2$
C05	DACSKQGMGVLLSGWPGPCTT	1	X_2CX_{18}
C07	PACLLRSEEYVVECGGDVGLE	1	X_2CX_{18}

Biochemistry, 38:12499

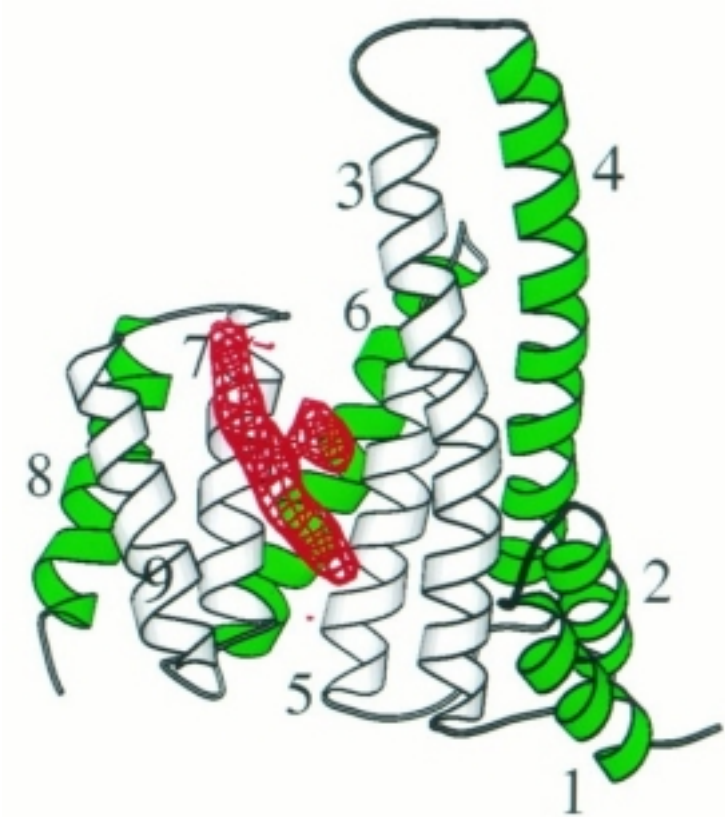
R18 Peptide Inhibits Interaction Between 14-3-3 and Raf-1



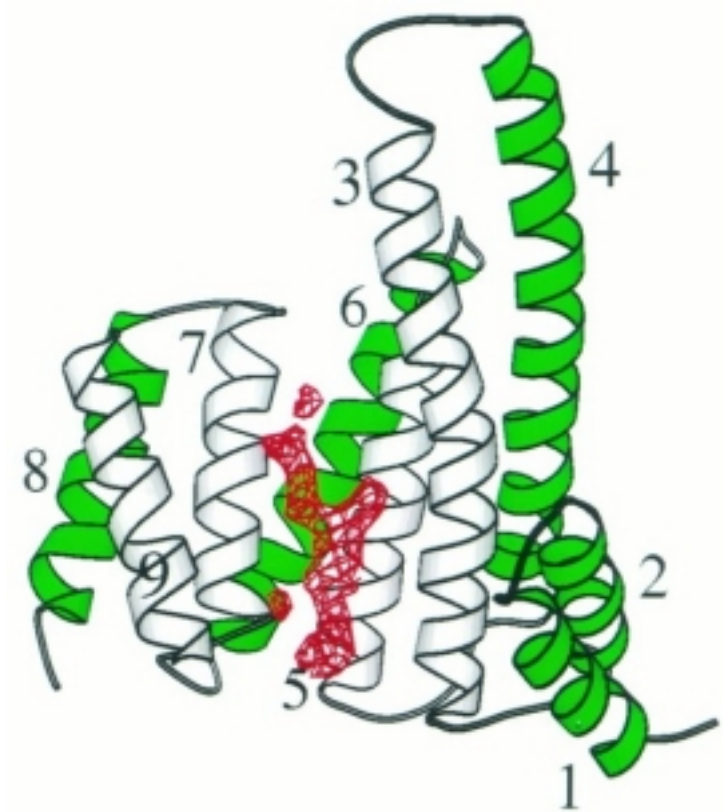
Method:
Far-Western blot of total cell lysate with ^{125}I-labeled 14-3-3ζ.

*GST fusion peptide worked as wells as synthetic peptide.

R18 and Native Ligand Target Overlapping Binding Pocket



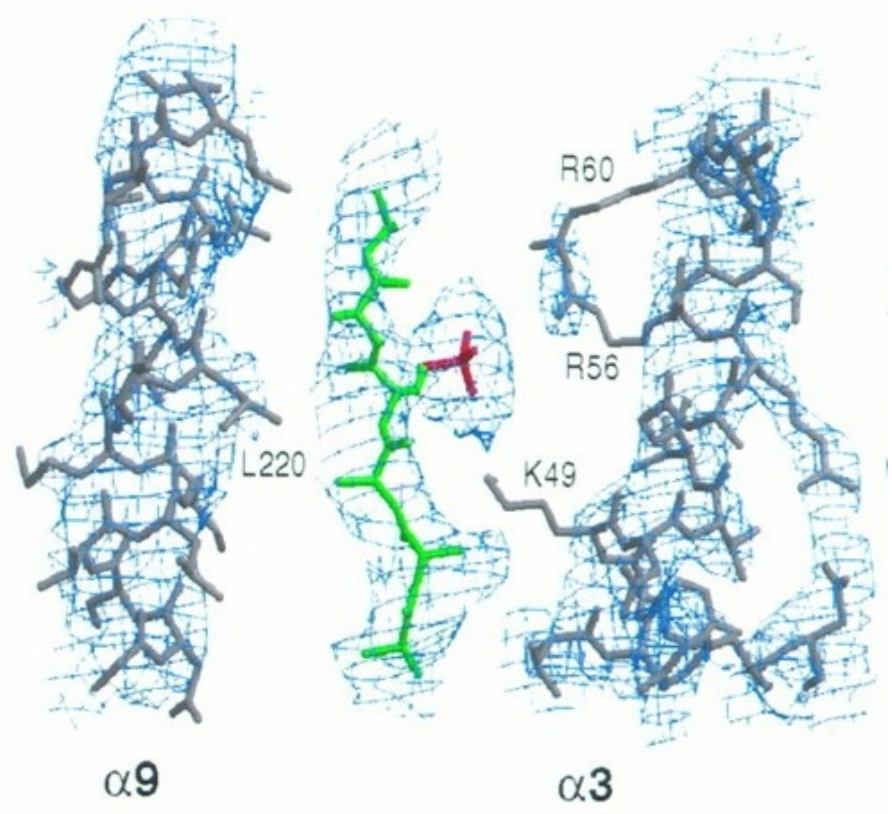
Raf (RSTpSTPN)



R18 (WLDLE)

Collaboration with R. Linddington and H. Fu

R18 Utilizes Acidic Amino Acids to Mimic Phosphoserine



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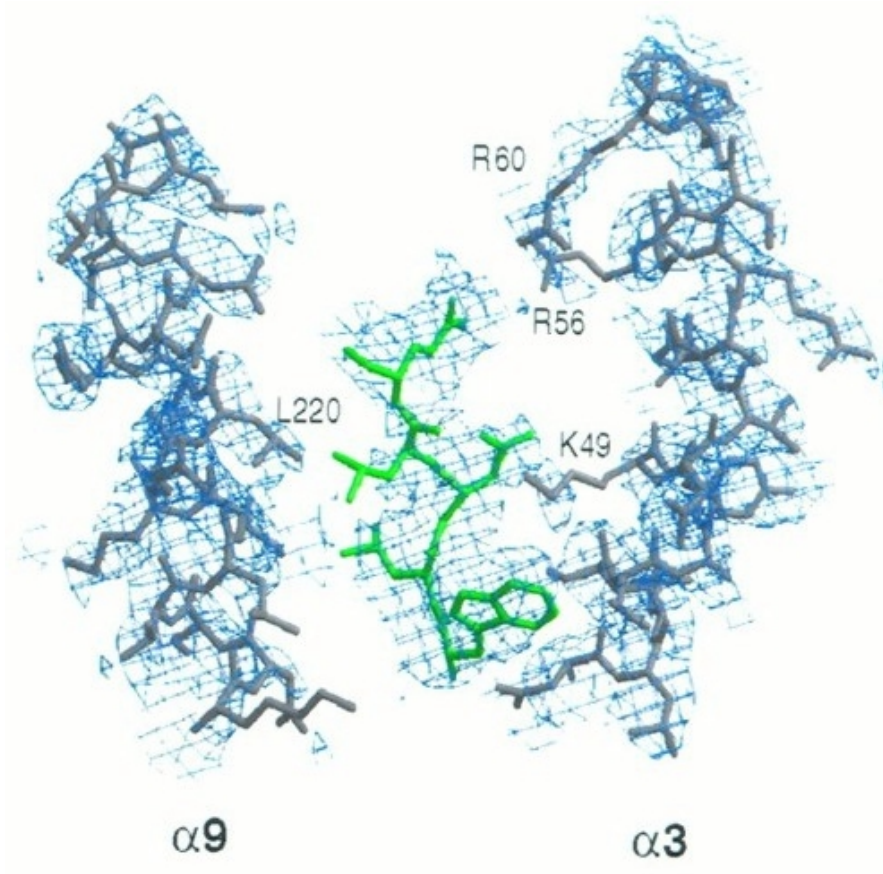

R18 (WLDLE)

Affinity-Selection of 14-3-3ζ Ligand Displayed on pIII of M13 Phage

Clone	Sequence	Frequency	Library
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C43	VTCGSIAEYGWLDLAAACSS	1	$X_2CX_{14}CX_2$
C12	PRCMQTSYWMDGLQPESCKG	1	$X_2CX_{14}CX_2$
C48	RNCWGNIPLTSSSVERLCDAR	1	X_2CX_{18}
C03	RVCAAPESRLFRGMPLGCDD	1	$X_2CX_{14}CX_2$
C05	DACSKQGMGVLLSGWPGPCTT	1	X_2CX_{18}
C07	PACLLRSEEYVVECGGDVGLE	1	X_2CX_{18}

Biochemistry, 38:12499

X-ray crystallography of R18 peptide-14-3-3ζ complex:

- Peptides isolated from phage display libraries almost invariably target ligand-binding pockets, or hot spots, of ligates.

- They usually model three dimensional interaction interface of the native ligands, rather than linear sequence. One can not simply blast database for native ligands based on peptide sequences.

Applications and Expected Outcomes

1) Biopanning on recombinant proteins.

14-3-3ζ-binding peptide (R18), and crystallographic structure of R18-14-3-3ζ complex.

2) Biopanning on cells, a new strategy.

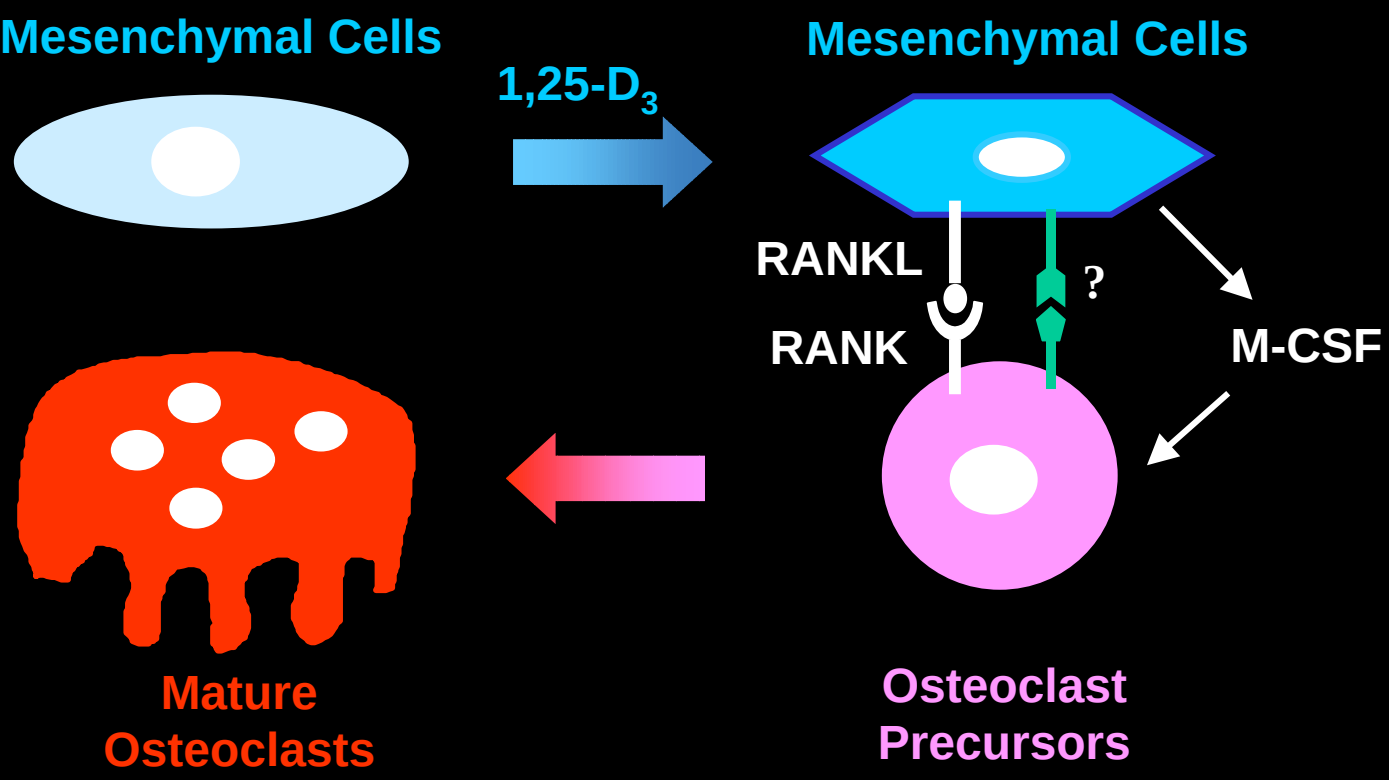
Differentiated vs undifferentiated cells, stimulated vs unstimulated, or transfected vs untransfected cells.

Problem: Cell surface ligates have been elusive to phage display.

3) Toward peptide-based therapy in vivo.

Inhibition of tumor growth by an EphA2 kinase binding peptide fused to IgG.

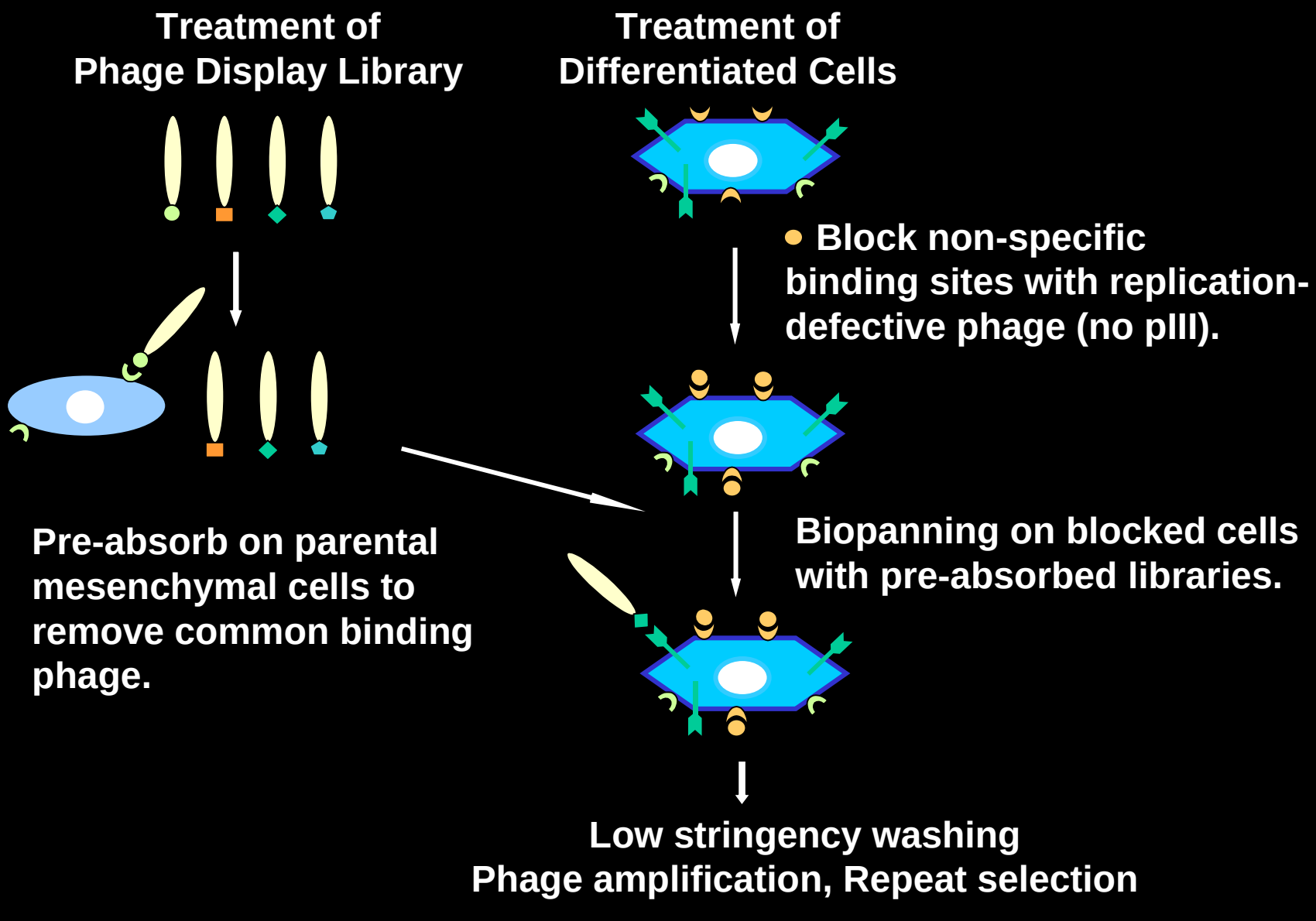
Osteoclast Differentiation Requires Contact with Mesenchymal Cells or Immature Osteoblasts



Hypothesis: Peptide ligands that bind to 1,25-D3-induced ligates on mesenchymal cells may inhibit osteoclast differentiation.

Collaboration with E. Greenfield

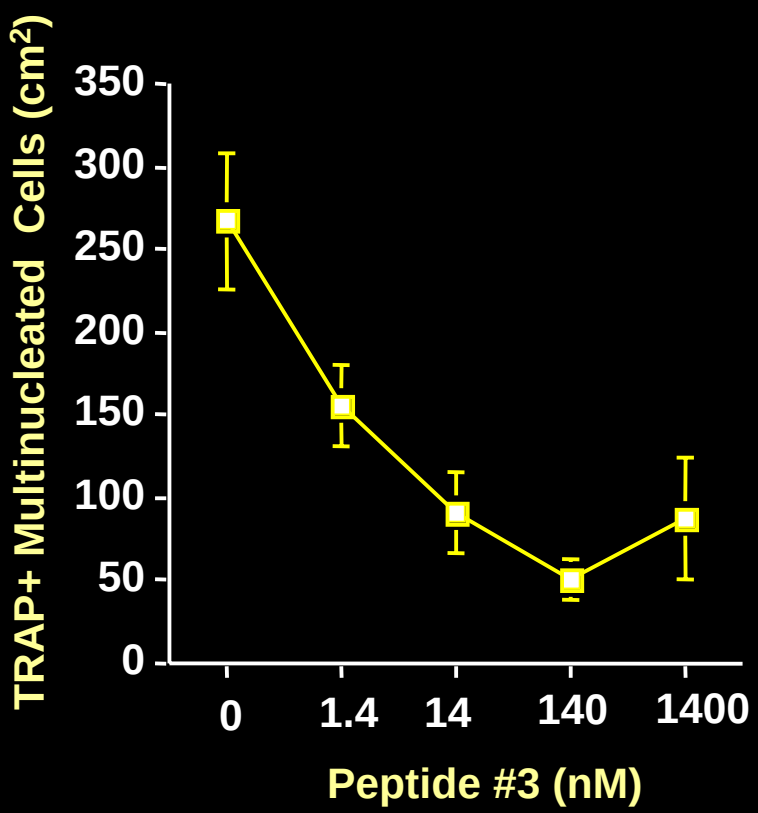
A New Strategy for Cell Surface Biopanning

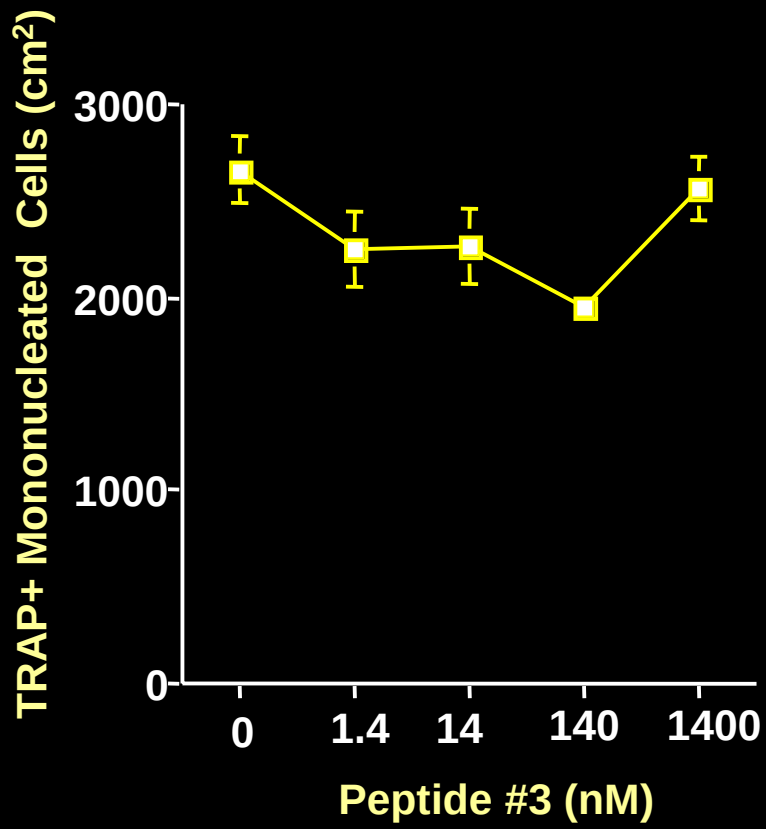


Sequence Analysis of Phage Clones That Specifically Bound to 1,25-D-Treated but not Untreated Mesenchymal cells

Phage Families	No. of Clones	Library
1	50%	$X_2CX_{14}CX_2$
2	27%	$X_2CX_{14}CX_2$
3	7%	X_2CX_{18}
4	17%	X_2CX_{18}

An Osteoblast-Binding Peptide From Family 3 Inhibited Osteoclast Differentiation





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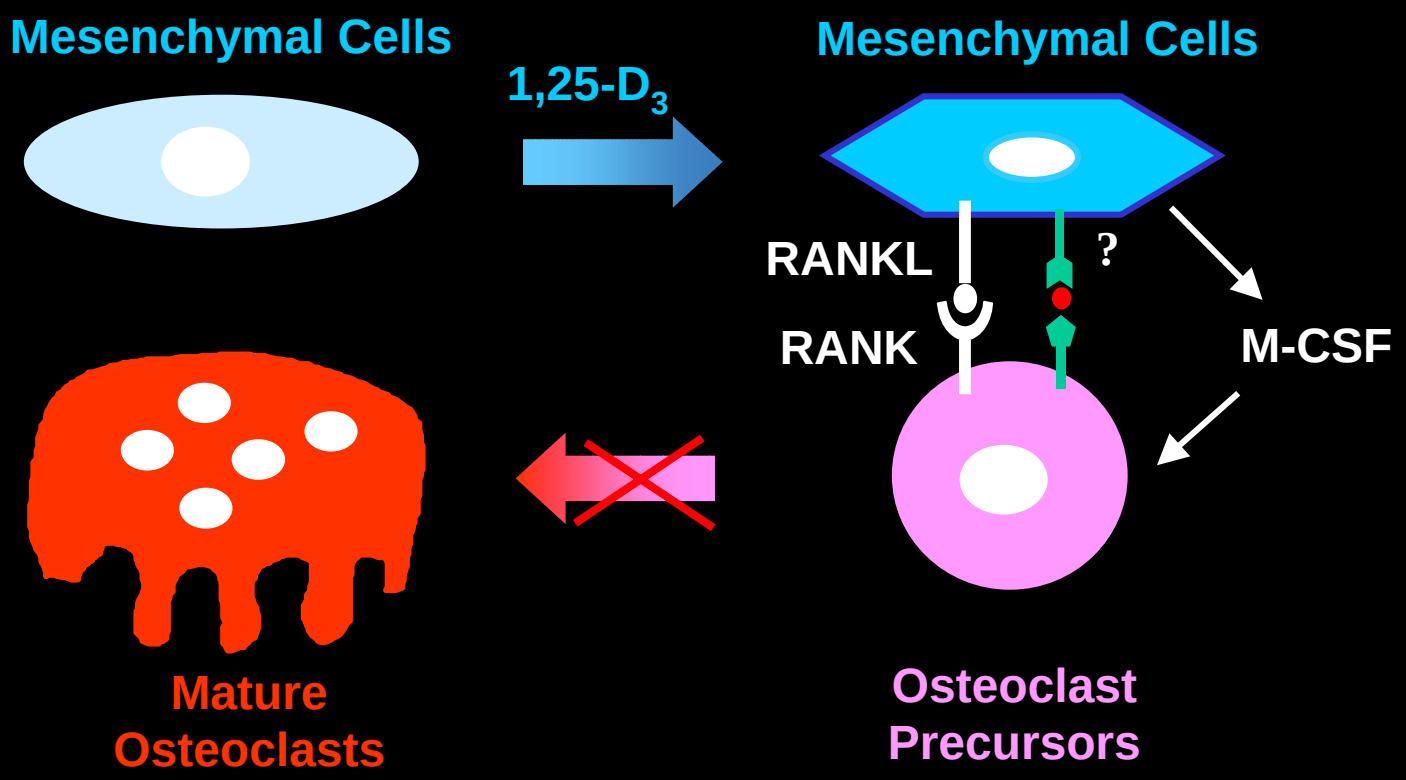

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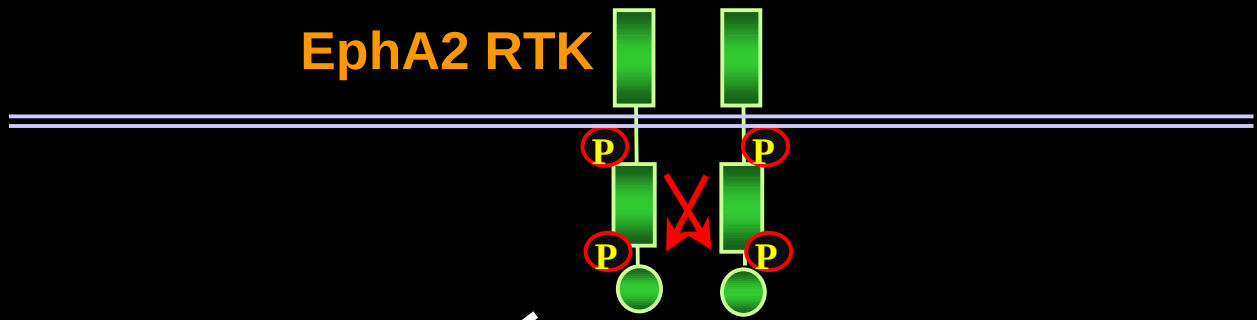
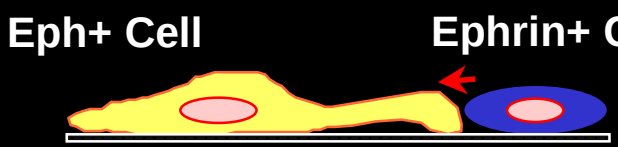
   Inhibition of tumor growth by an EphA2 kinase binding peptide fused to IgG.

EphA2 Ligation Activates Two Novel Signaling Pathways



Inhibition of Cell Spreading and Migration

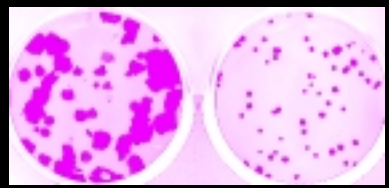
Repulsive Guidance of Migrating Cells



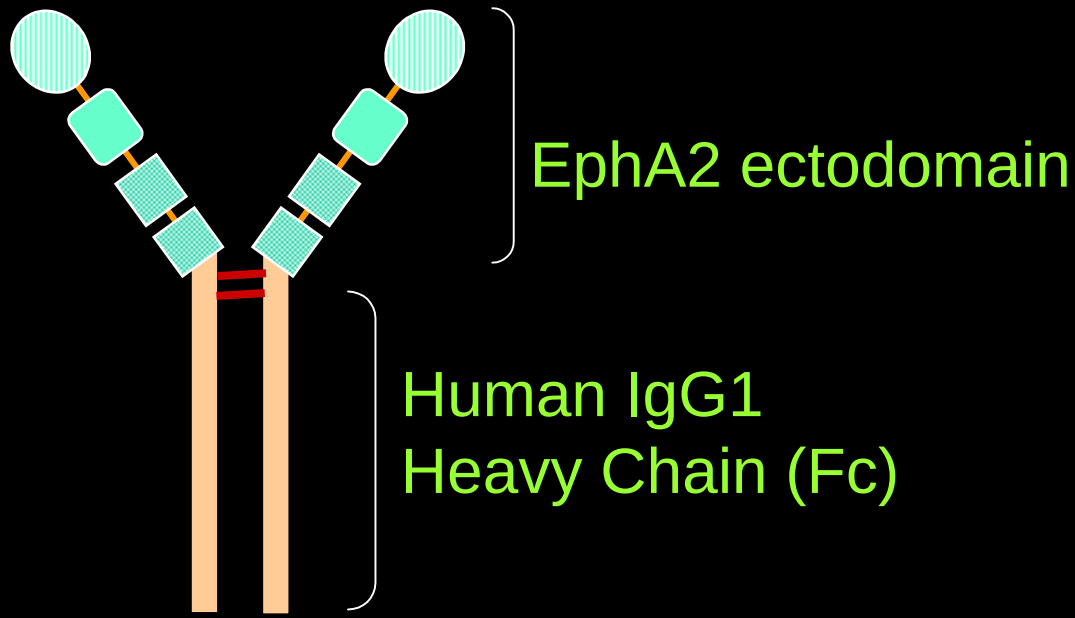
(Nature Cell Biol. 2:62)

Inhibition of Ras/MAPK cascade

Reduced cell proliferation



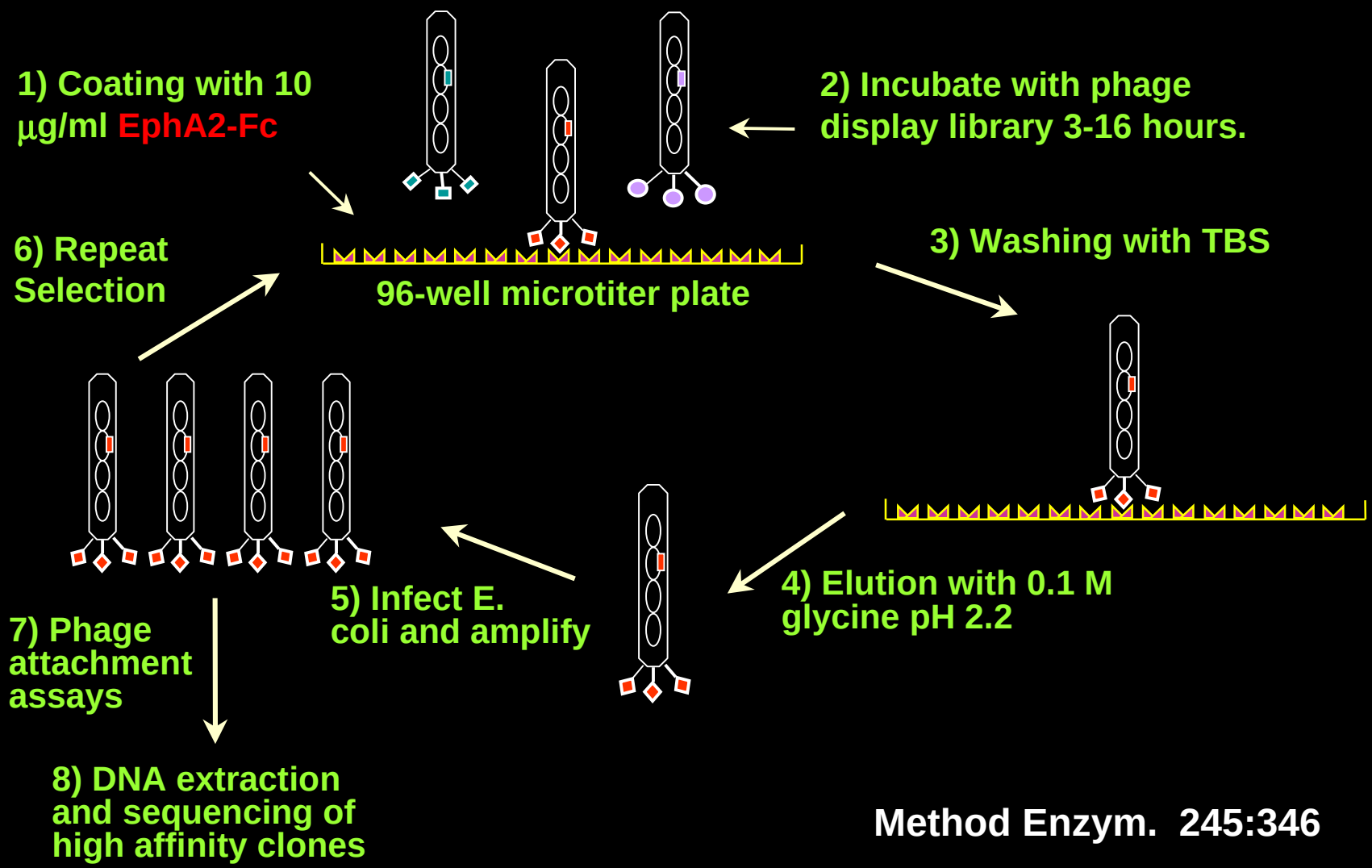
(Nature Cell Biol. 3:527)



EphA2-Fc

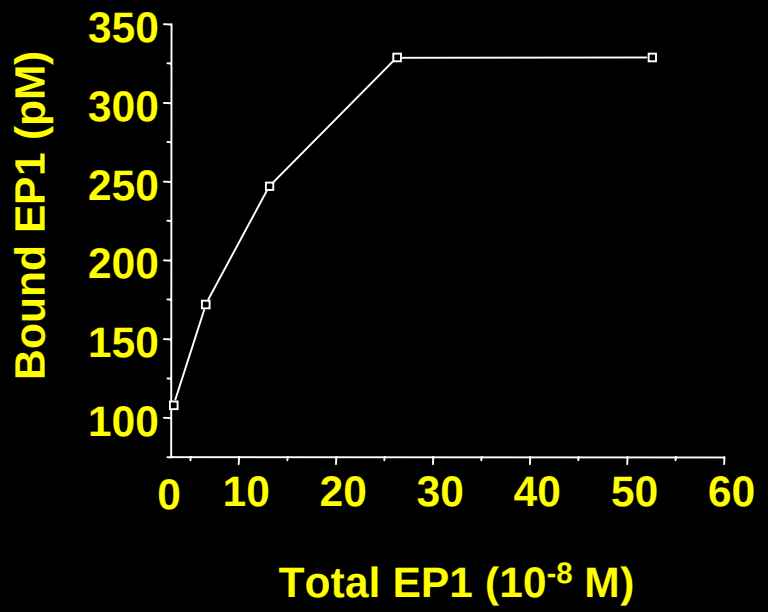
Produced as secreted proteins
in the stably transfected 293 cells.

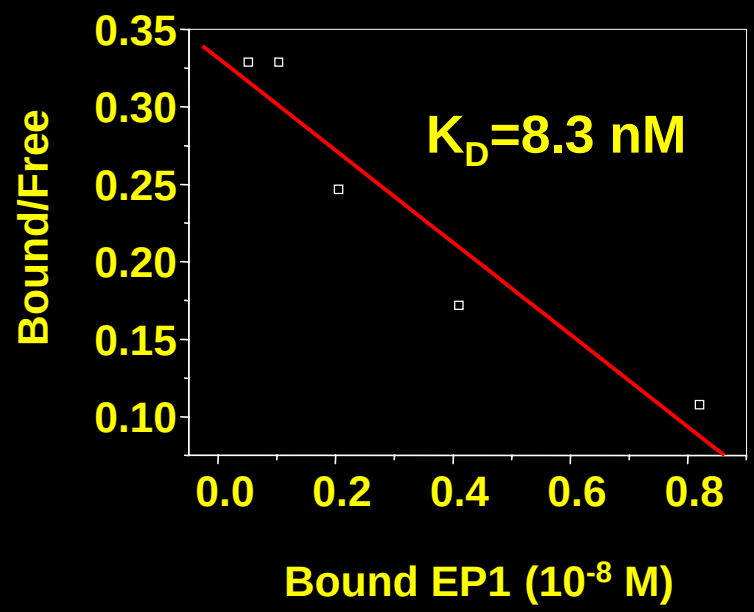
Searching Peptide Ligands on Immobilized GST-14-3-3ζ



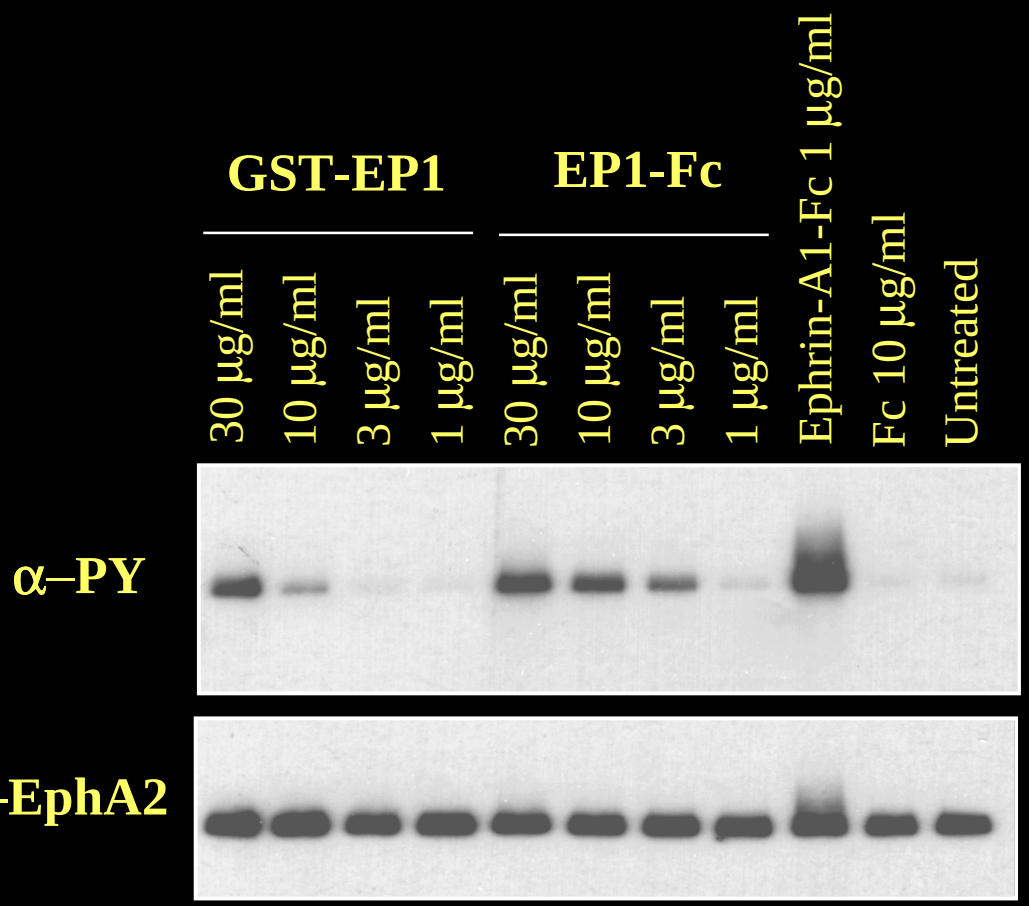
Method Enzym. 245:346

Kinetics of EP1 Peptide Binding to EphA2



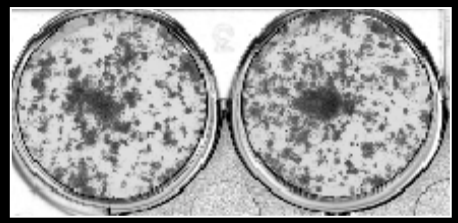


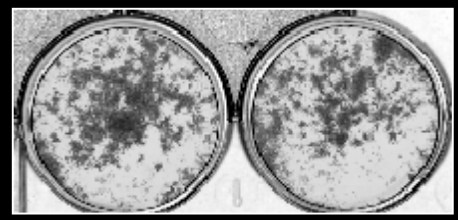
EP1 Peptide Is an Agonist of EphA2 Kinase in PC-3 Cells



EP1 Peptide Inhibits Prostate Epithelial Cell Proliferation

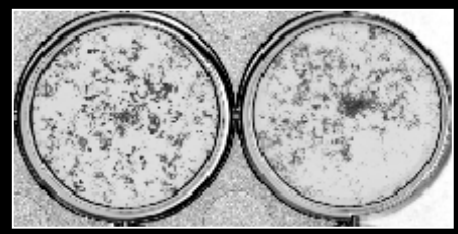
Untreated





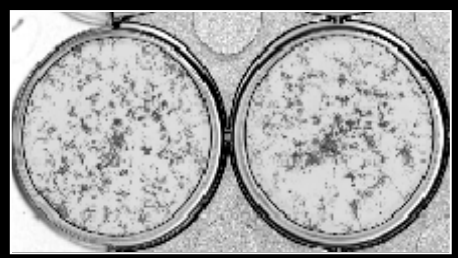
Fc
20 μg/ml

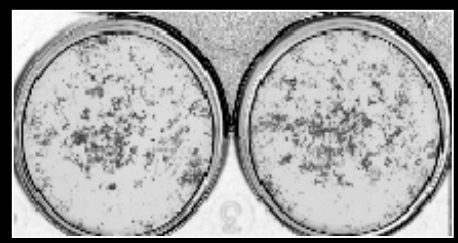
EphrinA1-Fc
0.5 μg/ml



EP1-Fc
5 μg/ml

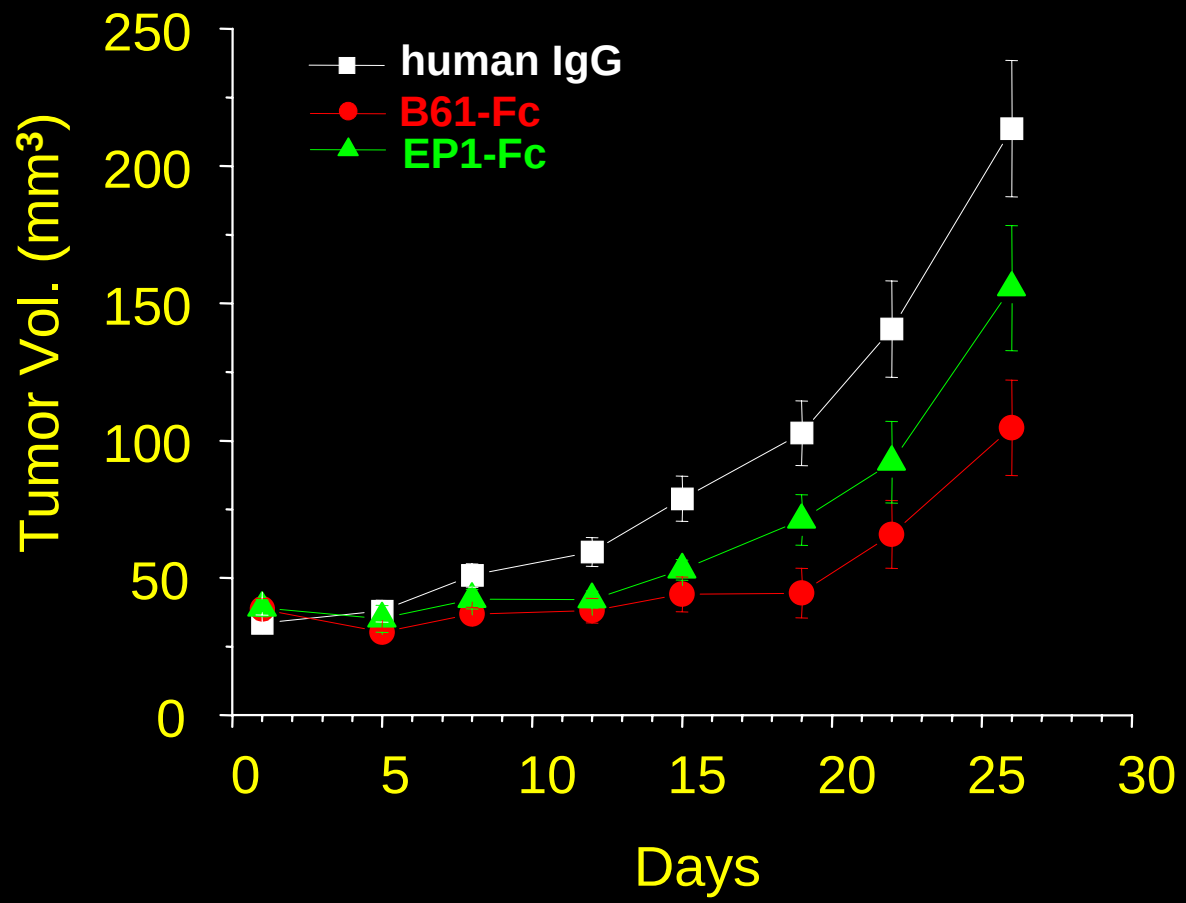
EphrinA1-Fc
2.0 μg/ml





EP1-Fc
20 μg/ml

Both Ephrin-A1-Fc and EP1-Fc Fusion Peptide inhibited Tumor Growth of the Subcutaneously-Injected PC-3 Cells in *nu/nu* Mice



Summary

1) Peptide phage display allows large scale (up to 10^{10}) sampling of peptide ligands for a given ligate.

2) Since phage display peptides selectively target the binding pockets, they can be valuable in identifying “hot spots” of protein-protein interactions.

3) With the new affinity-selection strategy, cell surface proteins can be readily targeted with peptide display libraries. Peptides may selectively bind to a single transfected protein, or they can represent a pool of peptides that target many known or unknown cell surface proteins in response to an experimental stimulus.

4) Human IgG-fusion peptides make in vivo testing of small peptides feasible. Indeed, the fusion peptides themselves may be directly or indirectly used in disease therapy.

Advantages

1) Low cost.
2) Requires no special resources.
3) Can be performed by small labs.
4) Quick sampling of large pools of ligands, in the absence of filter screening, or microarray.
5) Can mimic posttranaslational modification.
6) Ligands can be directly tested in vitro and in vivo.

Disadvantages

1) Reliance on representative primary libraries, which can be time-consuming to generate.
2) Ligands are functional mimetics rather than sequence mimetics. One can not predict native ligands based on peptide sequences. cDNA phage display to the rescue?
3) Does not work for all proteins. Switching to different libraries will help.
4) Competition with pharmaceutical giants?